## Supplemental Data

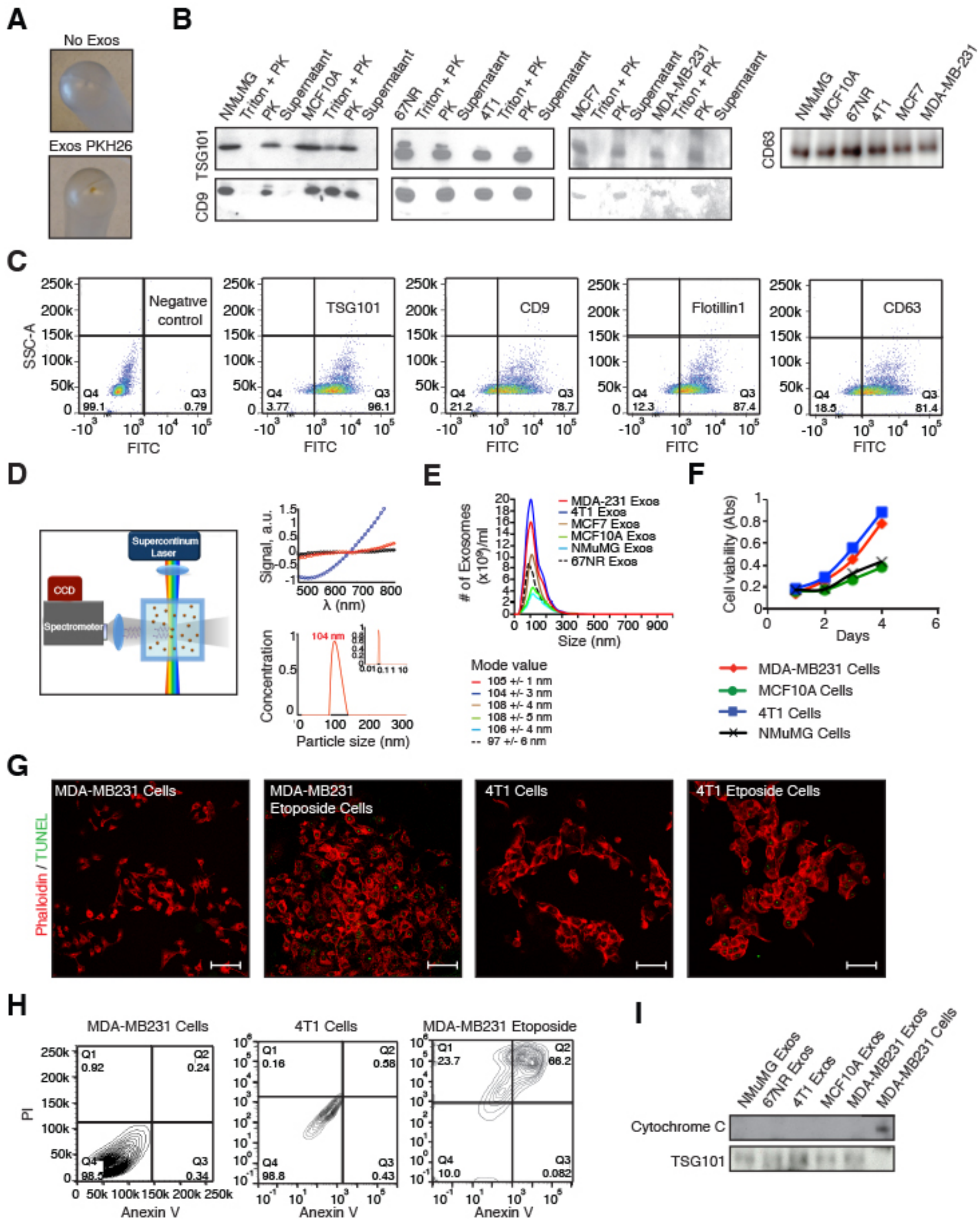
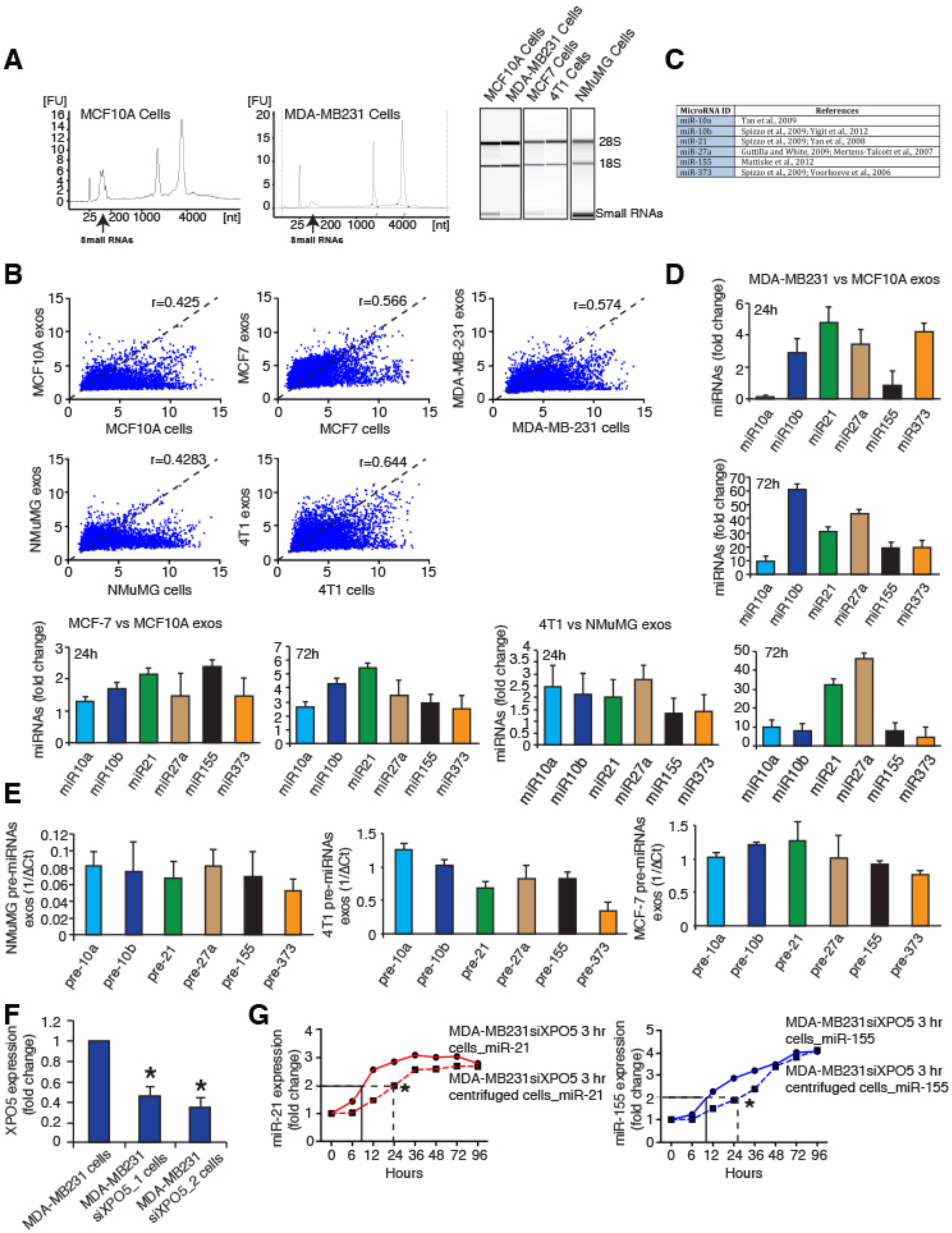


**Figure S1, Related to Figure 1. Cancer exosomes become enriched in miRNAs**

(A) Photograph of PKH26 stained exosomes, at the bottom of the ultracentrifugation tube. (B) Immunoblot for exosomes markers TSG101, CD9 and CD63. Exosomes extracts were treated with triton X + proteinase K or proteinase K alone. (C) Flow cytometry analysis using exosomes markers TSG101, CD9, flotillin-1 and CD63 antibodies of MDA-MB231-derived exosomes coupled to 0.4 μm beads. Negative control corresponds to secondary conjugated antibody. (D) Sizing exosomes with Light Scattering Spectroscopy (LSS). Schematic representation of experimental system used to collect LSS spectra (left image). Calibration of the system was done using signals from phosphate buffered saline (PBS) suspensions of glass microspheres with nominal diameters of 24 nm and 100 nm and polystyrene microspheres with nominal diameters of 119 nm, 175 nm, 356 nm and 457 nm. The experimental spectra and resulting fits are shown in the top graph for glass microspheres with nominal diameter of 100 nm and polystyrene microspheres with nominal diameter of 356 nm. Bottom graph represents the size measurement of a PBS suspension of MDA-MB-231 cancer exosomes. Inset shows same graph with a scale up to 10 μm to exclude potential contamination of our exosomes preparations with cells and cellular debris. (E) Exosomes size distribution-using NanoSight. The graph represents the size distribution of particles in solution showing an average of the mode size for all exosomes represented (MDA-MB-231, 4T1, MCF-7, MCF10A, NMuMG and 67NR) of 105 nm and also showing no peaks at larger sizes. (F) Cell viability measured by MTT assay during 5 days of culture of MCF10A, NMuMG, MDA-MB231 and 4T1 cells. Experiment was performed every cell passage to assure good culture conditions. (G) Confocal microscopy showing TUNEL assay. Left panels cells are labeled in red using phalloidin and are negative for TUNEL. Right panels shows positive controls of cells for TUNEL treated with etoposide, a known apoptotic drug. Scale bars represent 20μm. (H) Flow cytometry analysis for propidium iodide (PI) and Anexin V of MDA-MB231 and 4T1 cells. MDA-MB231 cells treated with etoposide were used as a positive control for apoptosis. (I) Immunoblot analysis of cytochrome C in exosomes using MDA-MB231 cells as a positive control and TSG101 as a loading control for exosomes.

The FACs, LSS, NanoSight, MTT, TUNEL data presented in this figure are the result of three independent experiments each with three replicates, and are represented as ± SEM. **Table S1, related to Figure 1, miRNAs profiling of MCF10A and MCF7 exosomes. Provided as an Excel file. Table S2, related to Figure 1, differentially expressed miRNAs between MCF10A and MDA-MB-231-derived exosomes. Provided as an Excel file. Table S3, related to Figure 1, miRNAs expression profiling of NMuMG and 4T1 exosomes. Provided as an Excel file. Table S4, related to Figure 1, miRNAs expression profiling of MCF7 exosomes culture for 72 hr versus MCF7 exosomes cultured for 24 hr. Provided as an Excel file. Table S5, related to Figure 1, miRNAs expression profiling of MDA-MB-231 exosomes culture for 72 hr versus MDA-MB-231 exosomes cultured for 24 hr. Provided as an Excel file. Table S6, related to Figure 1, miRNAs expression profiling of 4T1 exosomes culture for 72 hr versus 4T1 exosomes cultured for 24 hr. Provided as an Excel file.**




| MicroRNA ID | References |
|---|---|
| miR-10a | Tan et al., 2009 |
| miR-10b | Spizzo et al., 2009; Yigit et al., 2012 |
| miR-21 | Spizzo et al., 2009; Yan et al., 2008 |
| miR-27a | Guttilla and White, 2009; Mertens-Talcott et al., 2007 |
| miR-155 | Mattiske et al., 2012 |
| miR-373 | Spizzo et al., 2009; Voorhoeve et al., 2006 |

**Figure S2, Related to Figure 2. Cancer exosomes get depleted of pre-miRNAs**

(A) Bioanalyzer graphical representation depicted in fluorescence units (FU) per nucleotides (nt) (graphs) of the RNA content of human mammary MCF10A (non-tumorigenic) and MDA-MB231 (breast cancer) cell lines; and gel images (right image) of the RNA of MDA-MB-231, MCF7, 4T1, MCF10A and NMuMG cell lines (B) Scatterplots derived from miRNA array data comparing exosomes with cells of origin. Pearson correlation coefficient, r, is used as a measure of the strength of the linear relationship between the two samples. (C) Table with miRNAs IDs used for the study and references that demonstrate their role in cancer. (D) Exosomes harvested from 4T1, MCF-7, MDA-MB231, MCF10A and NMuMG cells were resuspended in DMEM media FBS-depleted and maintained in cell-free culture conditions for 24 and 72h. After 24 and 72h exosomes were recovered and 6 miRNAs were quantified by qPCR. Graphs show the fold change of each miRNA in cancer exosomes after cell-free culture for 24 and 72 hr relative to normosomes after 24 and 72 hr of cell-free culture, respectively. (E) Six pre-miRNAs corresponding to the mature miRNAs previously quantified were quantified by qPCR in NMuMG, 4T1 and MCF-7 exosomes. The inverse of the ΔCt value for each pre-miRNA was plotted. (F) XPO5 mRNA expression in MDA-MB231 cells with two transiently transfected siRNAs targeting XPO5 compared as a fold change to control cells. (G) MDA-MB231 cells were transfected with XPO5 siRNA constructs and miR-21 and miR155 expression was assessed at several time points 12 hr post-transfection (0, 6, 12, 24, 36, 48, 72 and 96 hr). As a comparison to show the effect of long centrifugation time periods MDA-MB231 cells transfected with XPO5 siRNA constructs were centrifuged at 4°C for 3 hr and put back in culture. MiR-21 and miR155 expression was assessed at several time points post-centrifugation (0, 6, 12, 24, 36, 48, 72 and 96 hr). Processing of pre-miR21 to miR-21 or pre-miR155 to miR-155 is delayed in centrifuged cells as a two fold in the respective miRNAs is delayed by at least 16 hr.

The qPCR data presented in this figure are the result of three independent experiments each with three replicates, and are represented as ± SEM.; significance was determined using T test (*$p<0.05$).

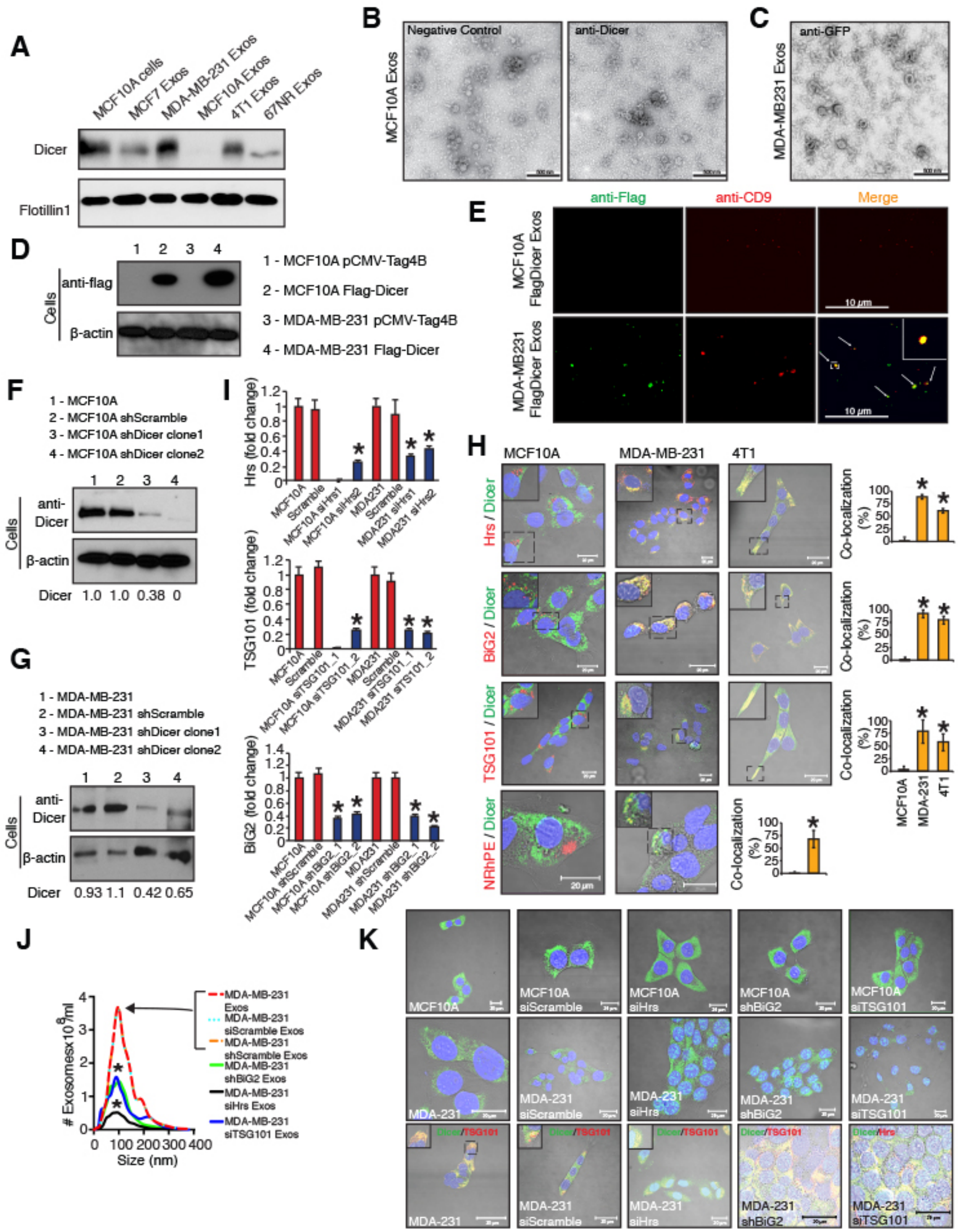


**Figure S3, Related to Figure 3. Cancer exosomes contain RLC proteins**

(A) Immunoblot of Dicer in 4T1, MDA-MB231 and MCF-7 exosomes. MCF10A cell lysate was used as a positive control and MCF10A exosomes as a negative control. (B) Transmission electron micrograph image produced by immunogold labeling using anti-Dicer antibody and negative control in MCF10A cells-derived exosomes. Negative control is secondary antibody. (C) Transmission electron micrograph image produced by immunogold labeling using anti-GFP antibody MDA-MB231-derived exosomes. (D) Immunoblot using anti-flag antibody (upper panel) in MCF10A and MDA-MB231 cells transfected with empty vector (pCMV-Tag4B; first and third lanes respectively) and Flag-Dicer vector (second and fourth lanes). Beta actin immunoblot was used as a loading control (lower panel). (E) Confocal microscopy images of exosomes harvested from normosomes (MCF10A upper panels) and cancer exosomes (MDA-MB231 lower panels) from cells transfected with Flag-Dicer. Immunodetection of Flag (first column; green staining) and CD9 (second column; red) proteins were performed and merged images show co-localization of Flag-Dicer and CD9 cancer exosomes (right lower panel; inset indicates zoomed image). (F) Immunoblot using anti-Dicer antibody (upper panel) in MCF10A, MCF10AshScramble and MCF10AshDicer clones 1 and 2, respectively (MCF10AshDicer clone1 and MCF10AshDicer clone2) cells. Beta actin immunoblot was used as a loading control (lower panel). (G) Immunoblot using anti-Dicer antibody (upper panel) in MDA-MB231, MDA-MB231shScramble and MDA-MB231shDicer clones 1 and 2 cells. Beta actin immunoblot was used as a loading control (lower panel). (H) Confocal microscopy images after immunocytochemistry for Dicer; early endosomes/MVBs components, Hrs and BiG2; late endosomes components, TSG101; in MCF10A, MDA-MB-231 and 4T1 cells and MVBs marker, NRhPE in MCF10A and MDA-

MB231 cells. Side graph of images represents the percentage of orange pixels in the image as quantified using image J software. Transmitted light was used to show the cells. (I) Hrs, TSG101 and BiG2 mRNA expression transfected with two different siRNAs for Hrs and TSG101 and two different sh clones for BiG2. (J) NanoSight particle tracking analysis of MDA-MB-231, MDA-MB-231siScramble, MDA-MB-231shSramble, MDA-MB-231siTSG101, -siHrs and shBiG2-derived exosomes showing down regulation of exosomes number in Hrs, TSG101 and BiG2 down regulated cells and the exosomes expected size distribution. (K) Confocal microscopy images after immunocytochemistry for Dicer in MCF10A and MDA-MB231 cells: transfected with siHrs (third column), shBiG2 (fourth column), and siTSG101 (last column). Controls of cells transfected with Scramble siRNA are shown in the second panels. Last row shows Dicer in green and multivesicular markers in red. Insets are zoomed images to show co-localization.

The qPCR data and the data presented in panels B, E, H, I and J of this figure are the result of three independent experiments, each with three replicates and are represented as ± SEM; significance was determined using T test (*$p<0.05$).

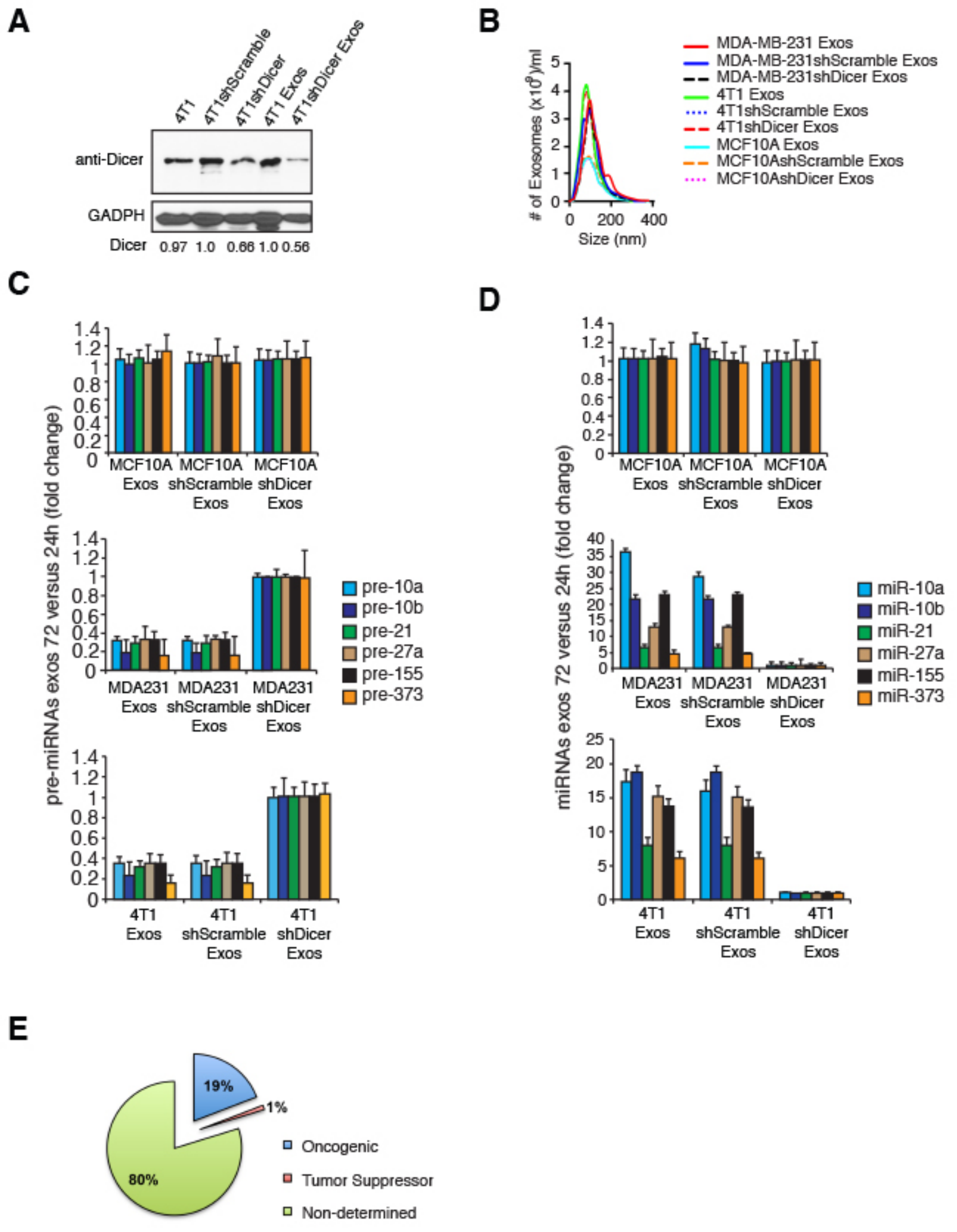



**Figure S4, Related to Figure 4. Cancer exosomes process pre-miRNAs to generate mature miRNAs**

(A) Immunoblot using anti-Dicer antibody in 4T1, 4T1shScramble and 4T1shDicer cells and exosomes harvested from 4T1 (4T1 exos) and 4T1shDicer

(4T1shDicer exos) cells (upper blot). GADPH immunoblot was used as loading control (lower blot). Quantification was done using Image J software. (B) Nanosight analysis of exosomes derived from MDA-MB-231, MDA-MB-231shScramble, MDA-MB-231shDicer, 4T1, 4T1shScramble, 4T1shDicer, MCF10A, MCF10AshScramble and MCF10AshDicer cells show no significant differences in size distribution or concentration of exosomes derived from parental cells or Dicer depleted cells. (C) Exosomes were harvested from MCF10A cells, MCF10A shScramble cells, MCF10A shDicer cells, MDA-MB-231 cells, MDA-MB-231 shScramble cells, MDA-MB-231shDicer cells, 4T1, 4T1shScramble and 4T1shDicer cells and maintained under cell-free culture conditions for 24 and 72 hr. After 24 and 72 hr, exosomes were extracted again and 6 pre-miRNAs (see **Figure S2C**) were quantified by qPCR. Graphs show the fold change of each pre-miRNA in the different exosomes after 72 hr of cell-free culture relative to 24 hr cell-free culture. (D) Exosomes were harvested from MCF10A cells, MCF10A shScramble cells, MCF10A shDicer cells, MDA-MB-231 cells, MDA-MB-231 shScramble cells, MDA-MB-231shDicer cells, 4T1, 4T1shScramble and 4T1shDicer cells and maintained under cell-free culture conditions for 24 and 72 hr. After 24 and 72 hr, exosomes were extracted again and 6 miRNAs (see **Figure S2C**) were quantified by qPCR. Graphs show the fold change of each miRNA in the different exosomes after 72 hr of cell-free culture relative to 24 hr cell-free culture. (E) Graphical representation of the categories (Oncogenic, Tumor Suppressor and Non-determined related to Cancer) of the down regulated miRNAs in MDA-MB231 exosomes electroporated with Dicer

(MDA-MB231 exos Dicer AB) compared to MDA-MB231 exosomes (MDA-MB231 exos). MicroRNAs were attributed to each category based on literature. The qPCR and NanoSight presented data in this figure are the result of three independent experiments each with three replicates and are represented as ± SEM. **Table S7, related to Figure 4, differentially expressed miRNAs between MDA-MB-231 exosomes and MDA-MB-231 exosomes with Dicer antibody. Provided as an Excel file.**

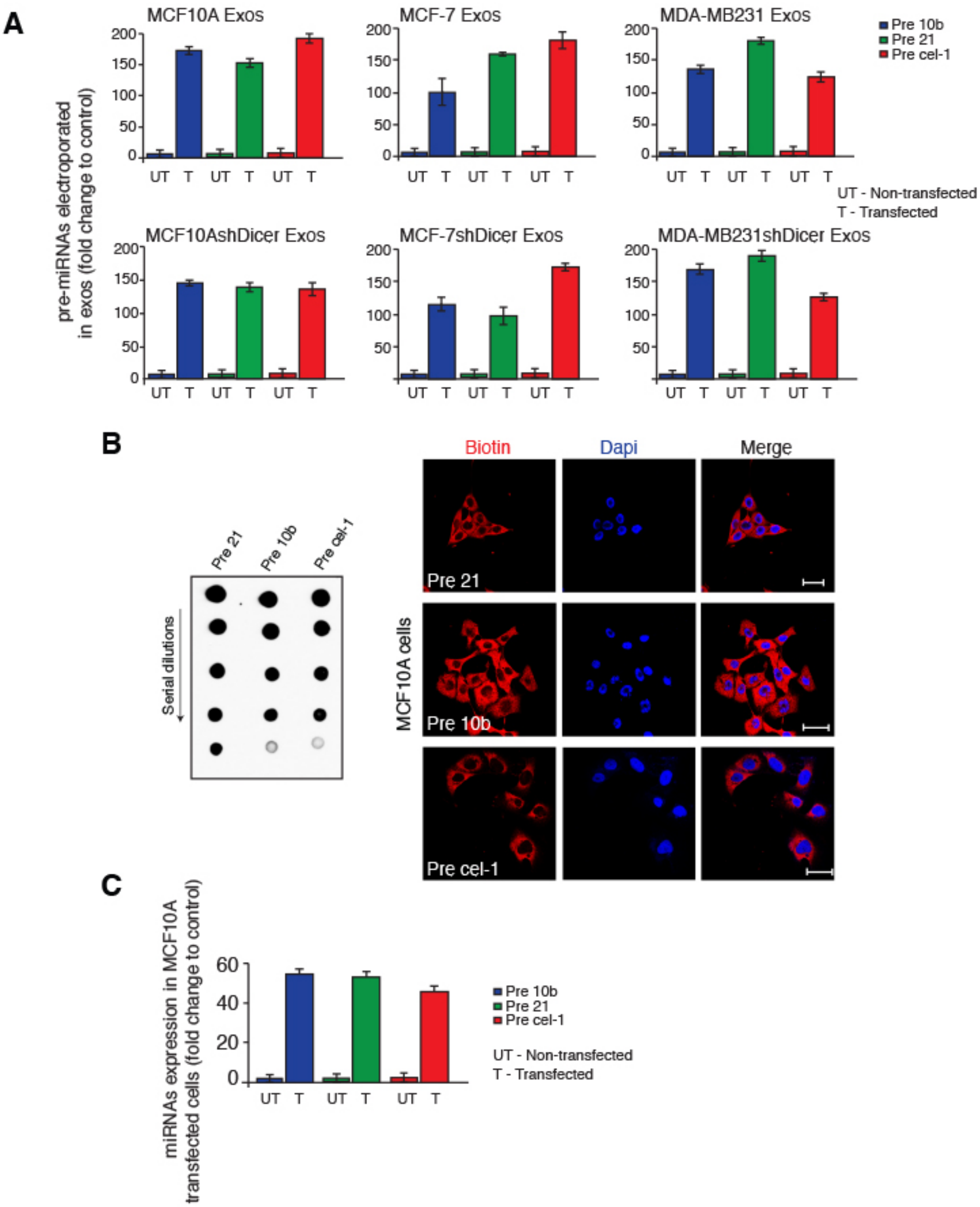



**Figure S5, Related to Figure 5. Cancer exosomes process pre-miRNAs to generate mature miRNAs**

(A) Exosomes were harvested from MCF10A, MCF10AshDicer, MDA-MB231, MDA-MB231shDicer, MCF7 and MCF-7shDicer cells and electroporated with synthetic pre-miRNA-10b, -21 and –cel-1. Each pre-miRNA was quantified by qPCR in the electroporated exosomes and represented as a fold change relative to exosomes that were electroporated with electroporation buffer only. (B) Dot blot of biotin internally labeled pre-miR-21, -10b and –cel-1 (left blot). Confocal microscopy images of biotin in MCF10A cells transfected with biotin internally labeled pre-miR-21 (first row), -10b (second row) and –cel-1 (third row). Scale bars correspond to 10, 20 and 20 μm, respectively. (C) miR-10b, -21 and –cel-1 expression analysis of MCF10A cells transfected with pre-miR-10b, -21 and –cel-1. Each bar represents the fold change of the transfected cells compared to non-transfected.

UT- Non-transfected; T – Transfected. The presented data in this figure are the result of three independent experiments each with three replicates and are represented as ± SEM.

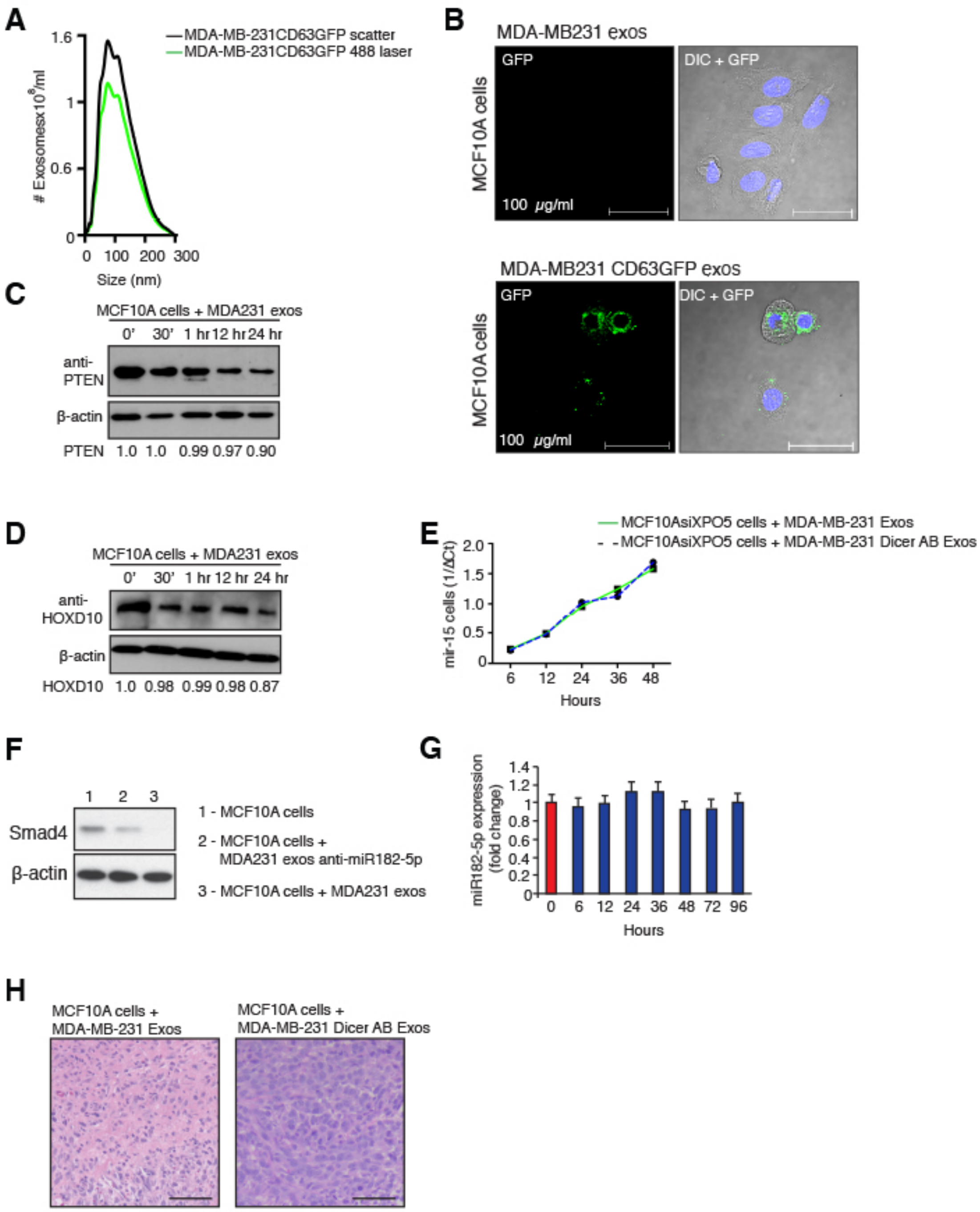



**Figure S6, Related to Figure 6. Cancer exosomes induce transcriptome alterations in recipient cells and tumor formation in a Dicer-dependent manner**

(A) NanoSight particle tracking analysis of exosomes derived from MDA-MB231 CD63-GFP cells. Black line represents a measure of total exosomes population and green line depicts the population of exosomes that is labeled with CD63-GFP using the NanoSight equipped with a 488 nm laser beam. (B) Confocal microscopy images of MCF10A cells treated with MDA-MB231 non-labeled exosomes (upper panels) and 100 µg/mL MDA-MB231 CD63GFP exosomes (lower panels). Scale bars are 50 µm. (C) Immunoblot using anti-PTEN antibody and protein extracts of MCF10A cells treated for 0, 30 min, 1, 12 and 24 hr with MDA-MB231 oncosomes freshly extracted. Beta actin was used as a loading control. (D) Immunoblot using anti-HOXD10 antibody and protein extracts of MCF10A cells treated for 0, 30 min, 1, 12 and 24 hr with MDA-MB231 oncosomes freshly extracted. Beta actin was used as a loading control. (E) MCF10A cells were transfected with siRNA for XPO5 to down regulate the flow of pre-miRNAs into the cytoplasm from the nucleus. The processing of pre-miR15 was assessed measuring the levels of miR-15 over time (6h, 12h, 24h, 36h and 48h) in MCF10AsiXPO5 cells and MCF10AsiXPO5 cells treated with MDA-MB231 exosomes with and without Dicer antibody. No significant changes were denoted. (F) Immunoblot using anti-Smad4 antibody (upper panel) and protein extracts of MCF10A cells and MCF10A cells treated with MDA-MB231 exosomes with anti-miR-182-5p and MDA-MB231 exosomes with no cell-free culture time. Beta actin was used as a loading control. (G) miR182-5p expression was monitored in MDA-MB231 derived exosomes over time (0, 6, 12, 24, 36, 48, 72 and 96 hr). Each bar represents the fold change of each time point compared to

0h. No significant differences were noted. (H) Hematoxylin-eosin (HE) staining of tumors arising from MCF10A cells co-injected into nude mice with MDA-MB-231 and MDA-MB-231-Dicer AB-derived exosomes (scale bars represent 100μm). Data are represented as mean ± SEM.

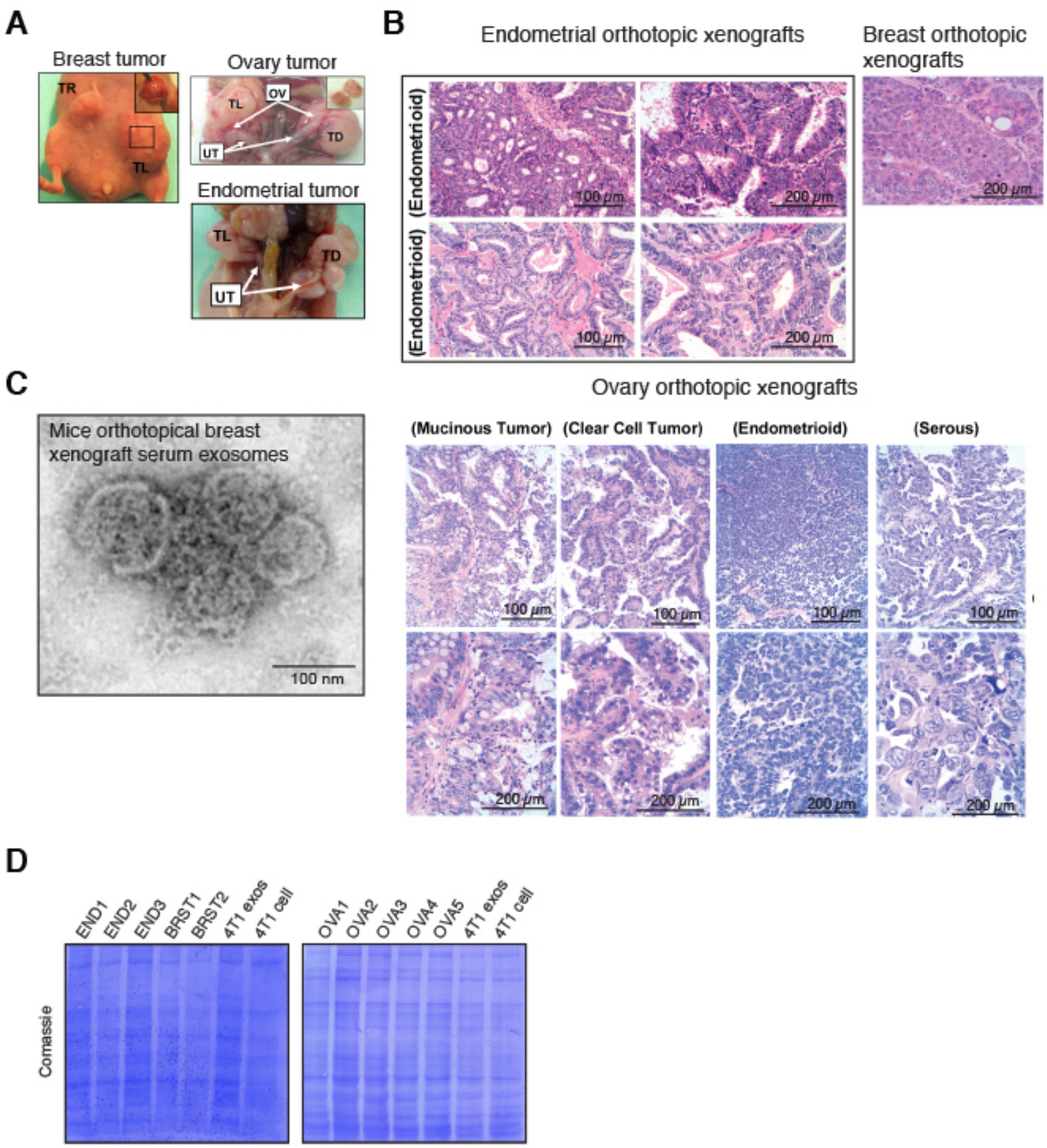



**Figure S7, Related to Figure 7. Serum from breast cancer patients contain Dicer and process pre-miRNAs**

(A) Representative photos from orthotopic xenografts derived from fragments of fresh primary human ovary, endometrial and breast tumors in nude mice. (B) Hematoxylin-eosin (HE) staining of ovary, endometrial and breast cancer orthotopic xenografts. (C) Transmission electron micrograph of serum exosomes harvested from mice with orthotopic tumor xenografts. (D) Comassie staining of membranes of immunoblots depicted in **Figure 7**A. Hematoxylin-eosin (HE) staining of tumors arising from MCF10A cells co-injected into nude mice with exosomes derived from breast cancer patients.

## Supplemental Experimental Procedures

### Isolation and purification of exosomes

Exosomes were purified by differential centrifugation as described previously[1,2]. Supernatant from cells cultured for 24 hr in media using exosomes-depleted FBS were subjected to sequential centrifugation steps of 800 g and 2000 g. The resulting supernatant was filtered using 0.2 μm filter in culture bottles. A pellet was recovered at 100,000g in an SW40Ti swinging bucket rotor after 2 hr (Beckman). Supernatant was removed and PBS was added to the pellet for a 1 hr washing step. The pellet was analyzed for exosomes. Exosomes used for RNA extraction were resuspended in 500 ul of Trizol; exosomes used for protein extraction were resuspended in 250 ul of lysis buffer (8 M Urea/2.5%SDS, 5 μg $ml^{-1}$ leupeptin, 1 μg $ml^{-1}$ pepstatin and 1 mM phenylmethylsulphonyl fluoride); and exosomes used for treatments were re-suspended in PBS. Frozen serum samples were thawed on ice and 500 μl were added to 12 mL PBS and identical procedure was followed as described above. Exosomes purified by centrifugation were treated (37°C, 60 min) with 500 mg/mL proteinase K (Sigma-Aldrich) dissolved in RNase-free water followed by heat inactivation of the protease (60°C, 10 minutes). Exosomes were incubated (37°C, 15 min) with 2 mg/mL protease-free RNaseA (Sigma-Aldrich) followed by addition of 10X concentrated RNase inhibitor (Ambion). For exosomes treatment, exosomes were purified in duplicate and one sample was used for protein quantification.

**Flow cytometry analysis of exosomes**

Exosomes preparations (5–10 μg) were incubated with 5 μl of 4-μm-diameter aldehyde/sulfate latex beads (Interfacial Dynamics, Portland, OR) and resuspended into 400 μl PBS containing 2% BSA. Exosomes-coated beads (20 μl) were incubated with the following antibodies: anti-CD63 (Santa Cruz), anti-CD9 (abcam), anti-TSG101 (abcam), anti-flotillin-1 (Santa Cruz) for 30 min at 4°C followed, when needed, by incubation with FITC-conjugated secondary antibody and analyzed on a FACS Calibur flow cytometer (BD Biosciences).

**Electroporation of exosomes**

Exosomes at a total protein concentration of 100 μg (measured by Bradford Assay) and 5 μg of Dicer antibody (polyclonal SC-30226, Santa Cruz, CA) or 5 μg of Actin antibody or 10 μg of pre-miRNA-21, -10b and –cel1, were mixed in 400 μl of electroporation buffer (1.15 mM potassium phosphate pH 7.2, 25 mM potassium chloride, 21% Optiprep). Exosomes were electroporated using a 4 mm cuvette using a Gene Pulser Xcell Electroporation System (BioRad), as previously described[3]. After electroporation, exosomes were treated with proteinase K and/or RNAse when appropriate.

**Light Scattering Spectroscopy (LSS)**

LSS spectra were collected using the experimental system described in Figure 2B. The Fianium SC-450-2 broadband supercontinuum laser was used as a source of white light. The light from the supercontinuum laser was focused into the sample with a long focus lens. The samples consisting of liquid suspensions of either exosomes or microspheres were placed in a custom cubic-shaped quartz sample holder. The background signals were collected from the solvent samples with no exosomes or microspheres. The light scattered by exosomes or microspheres at 90° to the incedent beam was collected with the other long focus lens and delivered to the Princiton Instrument Acton 2300i imaging spectrograph coupled with a high efficiency Andor Technology iXon DV885 EMCCD detector. The detection was performed in the 470–870nm wavelength range. The detector was controlled by a computer, into which the data were transferred, stored, and processed.

To calibrate the system and establish its ability to accurately detect particle sizes, which can be smaller than the wavelength, the signals from phosphate buffered saline (PBS) suspensions of glass microspheres with nominal diameters of 24 and 100 nm and polystyrene microspheres with nominal diameters of 119, 175, 356 and 457 nm were measured. The spectra predicted by Mie theory were fitted to the data using the previously developed least-squares minimization method. The experimental spectra and resulting fits are shown in Figure 1B for glass microspheres with nominal diameter of 100 nm and polystyrene microspheres with nominal diameter of 356 nm. Here, the deviation from the Rayleigh scattering multiplied by the fourth power of the wavelength is shown to emphasize the non-Rayleigh behavior of the LSS spectra. By comparing LSS yielded size distributions for microspheres with the manufacturer provided specifications, it was concluded that the accuracy of the LSS method is estimated to be 10 nm. It was also established that the reconstructed size distributions were insensitive to the refractive indices of the microspheres and the solvent. It should be pointed out that since light scattering of small particles is proportional to the six power of their size, detection of particles smaller than 50 nm in the presence of larger particles would require a substantial increase in the signal-to-noise ratio of the experimental system.

LSS experiments with the PBS suspension of exosomes were then performed. The experimental LSS spectrum of the exosomes and the corresponding Mie fit are presented in Fig. 1b. The fit of the reconstructed spectrum is excellent. Using the mentioned above reconstruction technique the size distribution of exosomes (see Fig. 1b right graph and insert), which peaked at 104 nm was found. This extracted size distribution was compared with the morphometric measurements performed on the TEM photographs of similar exosomes samples (Fig. 1a). Since number of particles on the TEM photographs was not large enough to plot a statistically meaningful distribution, the mean size of the particles larger than 50 nm was calculated from the TEM photograph and found to be equal to 95 nm. Thus, the LSS reconstructed size distribution and morphometric measurements performed on the TEM photographs of exosomes agreed with all the data.

**N-Rh-PE Treatments**

Cells were labeled with N-Rh-PE by incubating them with 8 μM N-Rh-PE (Avanti Polar Lipids, Alabaster, AL) diluted in ice-cold 1 X Hanks buffer (Invitrogen, Carlsbad, CA) for 1 hr on ice. Cells were washed 3 times with ice-cold Hanks buffer before plating them back in DMEM medium. N-Rh-PE cells were used for confocal imaging approximately 24 hr after labeling.

**Immunogold Labeling and Electron Microscopy**

Fixed specimens at an optimal concentration were placed onto a 300 mesh carbon/formvar coated grids and allowed to absorb to the formvar for a minimum of 1 minute. For immunogold staining the grids were placed into a blocking buffer for a block/permeablization step for 1 hr. Without rinsing, the grids were immediately placed into the primary antibody at the appropriate dilution overnight at 4°C (polyclonal anti-Dicer 1:10 SC-30226, Santa Cruz; monoclonal anti-CD9 1:10, Abcam). As controls, some grids were not exposed to the primary antibody. The next day all of the grids were rinsed with PBS then floated on drops of the appropriate secondary antibody attached with 10nm gold particles (AURION, Hatfield, PA) for 2 hours at room temperature. Grids were rinsed with PBS and were placed in 2.5% Glutaraldehyde in 0.1M Phosphate buffer for 15 minutes. After rinsing in PBS and distilled water the grids were allowed to dry and stained for contrast using uranyl acetate. The samples were viewed with a Tecnai Bio Twin transmission electron microscope (FEI, Hillsboro, OR) and images were taken with an AMT CCD Camera (Advanced Microscopy Techniques, Danvers, MA).

**Protein Blot and Antibodies**

To monitor the endogenous gene response, cells were harvested in RIPA buffer and exosomes in 8 M Urea/2.5%SDS, 5 μg $ml^{-1}$ leupeptin, 1 μg $ml^{-1}$ pepstatin and 1 mM phenylmethylsulphonyl fluoride buffer. Proteins were loaded according to Bradford quantification onto acrylamide gels and transferred onto PVDF membranes (ImmobilonP) by wet electrophoretic transfer. For protein samples of serum exosomes collected from the orthotopic xenograft models, a 4% acrylamide gel with 15 cm height was used to resolve human and mouse Dicer bands. Blots were blocked for 1hr at RT with 5% non-fat dry milk in PBS/0.05% Tween and incubated overnight at 4°C with the following primary antibodies: 1:500 anti-Dicer (SC-30226, Santa Cruz); 1:1000 anti-Ubiquitinylated proteins, clone FK2 (Millipore); 1:500 anti-Flag M2-Peroxidase Clone M2 (Sigma); 1:500 anti-CD43 ab9088 (Abcam); 1:500 anti-PTEN, ab32199, (Abcam); 1:300 anti-CD9 ab92726, (Abcam); 1:500 anti-GADPH ab9483, (Abcam); 1:250 anti-TRBP ab72110, (Abcam); 1:300 anti-TSG101 ab83, (Abcam); 1:400 anti-AGO2 ab32381, (Abcam); 1:4000 anti-β-actin Peroxidase Clone AC-15, (Sigma); 1:500 anti-GFP ab6556, (Abcam); 1:500 anti-HOXD10 ab76897 (Abcam). Secondary antibodies were incubated for 1hr at RT. Washes after antibody incubations were done on an orbital shaker, four times at 10 min intervals, with 1X PBS 0.05% Tween20. Blots were developed with chemiluminescent reagents from Pierce.

**Real-time PCR Analysis**

DNase treated RNA was retro-transcribed with MultiScribe Reverse Transcriptase (Applied Biosystems) and oligo-d(T) primers following total RNA purification with Trizol (Invitrogen). Real-time PCR for mRNAs was performed on an ABI PRISM 7300HT Sequence Detection System Instrument using SYBR Green Master Mix (Applied Biosystems) and β-actin as the control. The primers are listed in the table below. Pre-miRNAs were quantified using 150 ng of DNase treated RNA and the SuperScript III Platinum One-Step RT-qPCR kit (Invitrogen)[4]. The primers are listed in the table below. For miRNA expression analysis, 10 ng of RNA was mixed with TaqMan MicroRNA Reverse Transcription Kit reagent containing specific miRNA primers and reverse-transcribed according to the manufacturer's instructions (Applied Biosystems). Reaction mixes were incubated at 16°C for 30 min, 42°C for 30 min and 85°C for 5 min. Real-time PCR was performed using ABI PRISM 7300HT Sequence Detection System Instrument (Applied Biosystems) using the commercially available Assay-on-Demand for each miRNA studied (Applied Biosystems). Expression of miRNAs was normalized to the expression of 18S rRNA (TaqMan Pre-Developed Assay Reagent; Applied Biosystems) that served as the internal control for the RNA amount and integrity. Each measurement was performed in triplicate. Threshold cycle[5], the fractional cycle number at which the amount of amplified target reached a fixed threshold, was determined and expression was measured using the $2^{-\Delta Ct}$ formula, as previously reported[6].

**qPCR Primers Sequence**

**Genes**

BiG2 F: 5’ CAGGAGGTGGTGAAGGACAT3’

BiG2 R: 5’ CCCGTTGGTCTGTGAGTTT3’

TSG101 F: 5’ GATACCCTCCCAATCCCAGT3’

TSG101 R: 5’ GTCACTGACCGCAGAGATGA3’

Hrs F: 5’AGTGGCTGTCGGGTATTCATC3’

Hrs R: 5’CCGTCCATATCCCTTGAAGAATC3’

CD43 F: 5’GCTGGTGGTAAGCCCAGAC3’

CD43 R: 5’GGCTCGCTAGTAGAGACCAAA3’

hsa-Actin F: 5’CATGTACGTTGCTATCCAGGC3’

hsa-Actin R: 5’CTCCTTAATGTCACGCACGAT3’

mmu-Actin F: 5’GGCTGTATTCCCCTCCATCG3’

mmu-Actin R: 5’CCAGTTGGTAACAATGCCATGT3’

**Pre-miRNAs**

Pre-miR-10a,b F: 5’TACCCTGTAGATCCGAATTTGTG3’

Pre-miR-10a,b R: 5’ATTCCCCTAGATACGAATTTGTGA3’

Pre-miR-21 F: 5’GCTTATCAGACTGATGTTGACTG3’

Pre-miR-21 R: 5’CAGCCCATCGACTGGTG3’

Pre-miR-27a F: 5’GCAGGGCTTAGCTGCTTG3’

Pre-miR-27a R: 5’GGCGGAACTTAGCCACTGT3’

Pre-miR-155 F: 5’GTTAATGCTAATCGTGATAGGG3’

Pre-miR-155 R: 5’GCTAATATGTAGGAGTCAGTTGGA3’

Pre-miR-373 F: 5’CTCAAAATGGGGGCGCTT3’

Pre-miR-373 R: 5’CACCCCAAAATCGAAGCACT3’

Pre-cel-1 F: 5’CCACCCCGTTCTACATACTTC3’

Pre-cel-1 R: 5’ACCGTACCGAGCTGCATACT3’

**Northern Blot**

Northern blot was performed using 3' Bio[TEG] DNA oligonucleotides of the reverse compliment to the mature miRNA as probes. Urea/acrylamide 15% gels were used to load 40 µg of exosomal RNA (DNase treated) together with 1X RNA loading dye after 2 minutes at 95°C, followed by a 2 min period on ice. MicroRNA marker was used according to manufacturer's instructions (N2102, New England BioLabs). Electrophoresis was conducted at 4°C for 3 hr using TBE 1X. Transfer was done using Whatman blotting papers and the BrightStar-Plus Positively Charged Nylon Membrane (Ambion) for 2 hr at 4°C with TBE 0.5X. The RNA was cross-linked to the membrane using a UV transilluminator for 20 min. Membranes were pre-hybridized by rotating for 1 hr at 42°C in Ambion's ULTRAhyb®-Oligo hybridization solution (Ambion). The probes were then thawed on ice and 150 ng were added per mL of hybridization buffer after 5 minutes incubation at 95°C, after which membranes were left in rotation overnight at 42°C. The following washes steps were done: 2X SSPE/0.5%SDS – twice for 15 minutes; 0.2SSPE/0.5%SDS – twice for 30 minutes and 2X SSPE - 5 minutes. These initial washing steps were followed by more washes then the blots were developed using the BrightStar BioDetect Kit according to the manufacturer's instructions (Ambion). The blots were exposed overnight with two stacked films. Blots were successfully stripped and re-probed twice more.

Northern probes:

miR-10b – 5'CACAAATTCGGTTCTACAGGG3';

miR-21 – 5' TCAACATCAGTCTGATAAGCTA3';

pre-miR-10b - 5'TGAAGTTTTTGCATCGACCATATATTCCCCTAGAATCGAA3';

pre-miR-21 – 5' TGTCAGACAGCCCATCGACTGGTGTTGCCATGAGAT3'

tRNA$^{Met}$–5'CAGCACGCTTCCGCTGCGCCACTCT3'

**Cell Culture, Plasmids, Pre-miRNAs and siRNAs**

MCF10A, MCF7, MDA-MB231, A549, SW480 and HeLa human cell lines, as well as NMuMG, 67NR and 4T1 mouse mammary cell lines were cultured in DMEM 10% FBS (all cells are originated from the American Type Culture Collection – ATCC). For exosomes extraction the culture media was replaced by FBS-depleted of exosomes 48 hr before collection of media. Transfections were performed using Lipofectamine 2000 reagent (Invitrogen) for siRNA. For synthetic pre-miRNA transfections RNAiFect (Qiagen) was used in all cell lines. See below for plasmids and siRNAs.

**Plasmids**

p-CMV-Tag4B-Dicer: a kind gift from Dr. Manel Esteller [7]; p-CMV6-CD63-GFP from Origene (RG217238); GFP-hAGO2 from Addgene (plasmid 11590); pGFP-shBiG2 from Origene (TG314697); pGFP-shDicer from Origene (TG304991); synthetic pre-miR-10b, -21 and –cel-1 were purchased from Ambion; 3'UTR-WT-PTEN, 3'UTR-Mutant-PTEN (Dr. Joshua Mendell laboratory), 3'UTR-WT-HOXD10 and 3'UTR-Mutant-HOXD10 (Dr. Robert Weinberg laboratory) are from Addgene.

**Sequences of siRNAs**

*HRS*: 5’GGAACGAGCCCAAGUACAATT3’ and

5’UUGUACUUGGGCUCGUUCCGG3’

*TSG101*: 5’GUUUAUCAUUCAAGUGUAATT3’ and

5’UUACACUUGAAUGAUAAACTG3’

*CD43*: 5’GGAGAGCCUUUGGUCUCUATT3’ and

5’UAGAGACCAAAGGCUCUCCGG3’

*AGO2*: 5’GGCGUUACACGAUGCACUUTT3’ and

5’AAGUGCAUCGUGUAACGCCTG3’

**Immunocytochemistry and Confocal Microscopy**

Cells were plated at an appropriate confluency in 12 well plates on inserted coverslips and cultured overnight. The next day cells were washed with cold PBS 1X and fixed for 20 min at RT with 4% PFA/PBS. Slides were permeabilized for 10 min at RT with PBS 0.5% Triton X-100, blocked 1 hr at RT with BSA 5%, and incubated overnight at 4°C with the following primary antibodies in PBST (PBS, 0.1% Triton) 2% BSA: 1:100 anti-Dicer (SC-30226, Santa Cruz); 1:500 anti-Flag (Sigma); 1:50 anti-CD43 ab9088 (Abcam); 1:100 anti-TSG101 ab83 (Abcam); 1:500 anti-GFP ab6556 (Abcam); 1:100 anti-LAPM-1 ab25630 (Abcam); 1:100 anti-Hrs ab56468 (Abcam); 1:100 anti-BiG2 ab75001 (Abcam); 1:500 anti-biotin ab66233 (Abcam). Secondary antibodies goat anti-rabbit Alexa 543 or goat anti-mouse Alexa-488 were incubated 1 hr at RT and diluted 1:200 in PBST 2% BSA. DAPI was used to stain the nuclei. For exosomes analysis, harvested exosomes were incubated with Triton X-0.05% for 15 min and subsequently with 5% BSA for 1 hr at RT. The first primary antibody (anti-CD9, 1:50) was incubated overnight in 100ul PBST at 4°C. The second primary antibody, anti-flag (1:50), was added the next day and incubated for 1 hr at RT. Secondary antibodies were added consecutively and incubated also for 1 hr at RT. Exosomes were plated on top of coverslips in 12 well plates in 4% PFA for 45 min and washed with cold PBS. Images were obtained using a Zeiss LSM510 Upright Confocal System using the recycle tool to maintain identical settings. Aggregated exosomes lead to structures larger than 200 nm that were visible in confocal microscopy. For data analysis, images were selected from a pool drawn from at least two independent experiments. Figures show representative fields.

### *In Vitro* Dicing Assays

Exosomal protein extracts (10 μg) were incubated at 37°C with 3 pmol of pre-miR-10b, -21 and -cel-1 biotin-internally labeled hairpins in the presence of 3 mM $MgCl_2$, 30 mM NaCl and 100 mM Hepes (pH 7.5). The final volume of each reaction was 10 μl. The reaction was stopped by the addition of 10 μl of formamide gel loading buffer. RNA was resolved using denaturating polyacrylamide gel electrophoresis and developed with the BrightStar BioDetect Kit according to the manufacturer’s instructions (Ambion).

### Cell Viability and Colony Formation Assays

Cells were plated in 96 well plates and harvested exosomes were added at day 1 at a concentration of 100 μg/mL. Cell viability was determined by the 3-(4,5-

dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. For colony formation experiments, cells were plated in 12-well plates and exosomes were added at day 1 and day 5 of culture at a concentration of 100 µg/mL. After 8 days, colonies were fixed and stained with MTT reagent.

**Illumina Human-HT12 mRNA Expression Array**

RNA was hybridized in an Illumina Human-HT12 mRNA expression array. Data was normalized using the neqc routine offered by the R package "limma" [8]. Gene abundances were determined by the median of the probes per gene. Clustering was done by arithmetic mean of euclidean distances of genes (rows) and samples (columns).

**miRNA expression array**

A custom miRNA array was used as described in[9]. The array contains 1833 human microRNA probes, 1084 mouse microRNA probes and other 78 non-coding RNAs probes. The probes are printed in duplicate. The GenBank accession ID associated with each probe is included. Bioinformatic analysis was performed using R (version 2.14.2) (http://www.r-project.org) and Bioconductor (http://www.bioconductor.org/). The raw intensity for each probe is the median feature pixel intensity with the median background subtracted. Setting an offset one ensures that there will be no negative values after log-transforming data. Data was quantile normalized followed by log2 transformation. Signals from probes measuring the same miRNA were averaged. The analysis was performed using the functions of LIMMA library. The heatmaps were generated using the heatplot function of made4 library. Heatmaps represent the average expression values obtained from replicate measurements.

**Orthotopic Xenografts of Ovary, Endometrium and Breast Tumors and Breast Cancer Serum Samples**

Female athymic nu/nu mice (Harlan) between 4 to 6 weeks of age were housed in individually ventilated cages on a 12 hr light-dark cycle at 21 to 23ºC and 40% to 60% humidity. Mice were allowed free access to an irradiated diet and sterilized water. All animal protocols were reviewed and approved according to the Spanish Institutional Animal Care and Use Committees.

The primary tumor specimens were obtained at the Hospital Universitari de Bellvitge (L'Hospitalet de Llobregat, Barcelona, Spain). The Institutional Review Board approved the study. Written informed consent was collected from patients. Non-necrotic tissue pieces (ca. 2–3 $mm^3$) from five representative resected human epithelial ovarian tumor (EOCs): serous, endometrioid, clear cell tumor and mucinous, were selected and placed in DMEM (BioWhittaker) supplemented with 10% FBS and penicillin/streptomycin at room temperature. Under isofluorane-induced anesthesia, animals were subjected to a lateral laparotomy, their ovaries exposed and tumor pieces anchored to the ovary surface with prolene 7.0 sutures. Additionally, pieces of human breast and endometrial tumors were implanted in the mammary fad pads and the endometrial wall, respectively.

Orthotopically engrafted tumors were allowed to grow and at the time of sacrificed 2 ml of blood were obtained from anesthetized mice by cardiac puncture. Samples were centrifuged at 14000 rpm and frozen at -80ºC.
Human breast cancer serum patient samples are collected and processed under the approved IRB protocol numbers: 04-0657 and 04-0698.

**Immunoprecipitation**

Cells and exosomes were harvested, washed in PBS and centrifuged or ultracentrifuged, respectively, to collect pellets. Ice-cold RIPA buffer or 8 M Urea/SDS buffer were added to cells and exosomes, respectively. Suspensions were gently rocked at 4°C, 15 min for cells and 2 hr for exosomes. The lysates were centrifuged at 14,000 g in a pre-cooled centrifuged for 15 minutes and the pellet was discarded. Protein A or G agarose/sepharose beads were washed twice with PBS and restored with a 50% slurry with PBS. A bead/slurry mix (100 μl) was added to 1 mL of cell lysate and 500 μl of exosomal lysate and incubated at 4°C for 10 min. Beads were removed by centrifugation at 14,000 x g at 4°C for 10 minutes and pellets discarded. Dicer antibody (5 μg for cells and 10 μg for exosomes) was added to 500 μl of cell lysate or 250 μl of exosomal lysate (1 μg/μl cells, 10 μg/μl exosomes) and incubated overnight at 4°C on an orbital shaker. 100 μl of Protein A or G agarose/sepharose bead slurry were added and left at 4°C overnight. After centrifugation the supernatant was discarded and beads washed 3 times with ice-cold RIPA buffer for cells or Urea/SDS buffer for exosomes. The agarose/sepharose beads were boiled for 5 minutes to dissociate the immunocomplexes from the beads. The beads were collected by centrifugation and protein blot was performed with the supernatant.

**Orthotopic injection of cells in nude mice**

Orthotopic tumor growth was measured by injecting MCF10A non-tumorigenic breast epithelial cells, MCF10A non-tumorigenic breast epithelial cells exposed to MDA-MB231-derived exosomes and MDA-MB-231 breast cancer cells ($1 \times 10^5$ cells in 0.2 ml PBS) into the mammary fat pad of 3-week-old female athymic nude mice, as described previously[10]. Tumor growth was monitored weekly by measuring the tumor length and width with a caliper and was reported as the mean tumor diameter as previously described[10]. All animals were euthanized 21 days post tumor cell injection.

## Supplemental References


1 Thery, C., Amigorena, S., Raposo, G. & Clayton, A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Current protocols in cell biology / editorial board, Juan S. Bonifacino ... [et al.]* **Chapter 3**, Unit 3 22, doi:10.1002/0471143030.cb0322s30 (2006).

2 Luga, V. *et al.* Exosomes Mediate Stromal Mobilization of Autocrine Wnt-PCP Signaling in Breast Cancer Cell Migration. *Cell* **151**, 1542-1556, doi:10.1016/j.cell.2012.11.024 (2012).

3 Alvarez-Erviti, L. *et al.* Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nature biotechnology* **29**, 341-345, doi:10.1038/nbt.1807 (2011).

4 Schmittgen, T. D., Jiang, J., Liu, Q. & Yang, L. A high-throughput method to monitor the expression of microRNA precursors. *Nucleic acids research* **32**, e43, doi:10.1093/nar/gnh040 (2004).

5 Rothstein, D. M. *et al.* Targeting signal 1 through CD45RB synergizes with CD40 ligand blockade and promotes long term engraftment and tolerance in stringent transplant models. *J Immunol* **166**, 322-329 (2001).

6 Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402-408, doi:10.1006/meth.2001.1262 (2001).

7 Melo, S. A. *et al.* A TARBP2 mutation in human cancer impairs microRNA processing and DICER1 function. *Nature genetics* **41**, 365-370, doi:10.1038/ng.317 (2009).

8 Thery, C. *et al.* Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes. *Nat Immunol* **3**, 1156-1162, doi:10.1038/ni854 (2002).

9 Liu, C. G., Calin, G. A., Volinia, S. & Croce, C. M. MicroRNA expression profiling using microarrays. *Nat Protoc* **3**, 563-578, doi:10.1038/nprot.2008.14 (2008).

10 Cosacov, A. *et al.* New insights into the phylogenetic relationships, character evolution, and phytogeographic patterns of Calceolaria (Calceolariaceae). *Am J Bot* **96**, 2240-2255, doi:10.3732/ajb.0900165 (2009).